Virulence and Diversity of *Blumeria graminis* f. sp. *hordei* in Israel and in the Czech Republic

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ABSTRACT

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Three hundred and nine isolates were obtained from three natural populations of *Blumeria graminis* f. sp. *hordei* occurring on wild barley (*Hordeum vulgare* subsp. *spontaneum*) at two locations in Israel during 1997 and 1999. Their virulence frequency was determined on 32 differential lines. No isolate was virulent on the differential lines possessing the genes *Mla13*, *mlo*, *Mlf1*, and *Mli*, and conversely no isolate was avirulent on the differential lines possessing the genes *MIRu2*, *MiLa*, *Mlh*, *Mla8*, *Mla25*, and *Mlj*. The frequencies of isolates overcoming the genes *Mlg*, *Mla7*, and *Mla27* were 0 to 16% at individual locations; frequencies of isolates overcoming the genes *Mla9*, *Mla17*, and *Mla18* ranged from 37 to 78%, and frequencies of virulences to genes *Mla1*, *Mla3*, *Mla6*, *Mlp1*, *Mlat*, *Mla12*, *Mlra*, *Mlk1*, *Mla19*, *Mla20*, *Mla26*, *Mla28*, *Mla29*, *Mla30*, *Mla32*, and *mlt1* were 79 to 99%. Based on examination of 376 isolates collected in the same years from the Czech Republic, these populations differed greatly from the Israeli ones. The Czech populations showed greater diversity of virulence and lower mean virulence complexity than the Israeli populations. Diversity in the Israeli populations differed also among clusters of niches at the same location.

Additional keywords: barley powdery mildew, *Erysiphe graminis* f. sp. *hordei*

Barley (*Hordeum vulgare* L.) is a minor crop in Israel, rarely exceeding an area of 1,500 ha. Most of it is grown in the more arid part of the country (northern part of Negev and the Jordan Valley). Spring barley is the only crop grown and is sown in late autumn (November to December) and harvested in spring (April to May). The cultivars are of local origin, and powdery mildew resistance has not been a selection criterion in breeding programs. Wild barley (*Hordeum vulgare* subsp. *spontaneum* (C. Koch.)) is not a predominant component of the wild plant communities, but it occurs widely in disturbed habitats throughout Israel. There are also particular localities where wheat is mainly grown and in which wild barley has become a very hard-to-control weed.

Barley powdery mildew, caused by *Blumeria graminis* DC. f. sp. *hordei* Ém. Marchal, is a windborne, biotrophic, fungal pathogen of cultivated barley and wild barley. It is particularly prevalent under

cool conditions when the maximum daily temperature does not exceed 25°C. The life cycle of the pathogen in Israel is closely linked with seasonal cycles (26). *B. graminis* f. sp. *hordei* survives summer as immature cleistothecia on dead leaves of barley until lower temperatures and autumn rains trigger the maturation of the cleistothecia and release of their ascospores. Maturation of cleistothecia coincides with the germination of barley seeds in the autumn. Epidemics of powdery mildew start from foci produced by the released ascospores, and develop via the conidial stage throughout winter and early spring. The change of day length at the beginning of spring induces cleistothecia formation. The pathogen remains dormant, as immature cleistothecia containing asci but no ascospores, until the next autumn. The sexual stage increases genetic diversity and is the source of primary inoculum. The population of *B. graminis* f. sp. *hordei* in Israel has co-existed and developed in association with wild barley rather than with cultivated barley.

Growing resistant cultivars is an economical and environmentally safe way of protecting barley against powdery mildew. Wild barley is a rich source of mildew resistance (10,14,21) and is cross-compatible with cultivated barley. The Fertile Crescent is the center of origin,

diversity, and domestication of wild barley, and many resistance genes are derived from wild barley originating from the Eastern Mediterranean. It is likely, therefore, that the pathogen has accumulated genes for virulence that will overcome resistance genes found in this region (6,7). Indeed, resistances found here are often ineffective against most pathogen strains found elsewhere (6,7). On the other hand, in parts of Europe where these resistance genes have recently been introduced into commercial cultivars, they are still effective. Therefore, barley breeders in Europe often use sources of resistance to powdery mildew from wild barley originating from Israel. The overall goal of this study was to verify the assumptions behind this practice by comparing virulence characteristics of *B. graminis* f. sp. *hordei* populations from Israel with other European populations, taking into account the fact that the Israeli populations infect diverse populations of wild barley. Specific objectives were: (i) to characterize Israeli *B. graminis* f. sp. *hordei* populations using standard European differentials (30) as well as standard barley lines carrying “new” resistance genes of wild barley to powdery mildew (20,21,23, 24,36); (ii) to compare these Israeli populations with populations from the Czech Republic; and (iii) to evaluate and assess diversity in and genetic distances among *B. graminis* f. sp. *hordei* populations by using various measures of genetic diversity and genetic distances. The Czech Republic was selected as representative of Europe because it is situated in the middle of the Central European part of the continent, barley is an important crop grown throughout the country with the second highest land area planted of all crops grown there, both spring and winter barley types are grown, and most cultivars grown possess the same resistance genes as those planted in neighboring countries. Czech *B. graminis* f. sp. *hordei* populations are expected to be similar to those in other countries in the Central European region because of pathogen migration in the region as well as cultivars with similar resistance genes.

MATERIALS AND METHODS

All tests for virulence of the Israeli populations were carried out at the Faculty of Agricultural, Food and Environmental

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Quality Sciences, Rehovot, Israel. All tests for virulence of the Czech populations were carried out at the Agricultural Research Institute Kroměříž Ltd. in the Czech Republic.

Location of Israeli *B. graminis* f. sp. *hordei* populations. Isolates of the pathogen were sampled at two locations. The first was at Ashkelon in a national park situated on the Mediterranean Sea coast, which is on the Southern Coastal Plain in Israel. This is a disturbed plant community, which contains many plants, like wild barley, that can swiftly colonize bare ground. The second location was at Har'el (Judean Foothills) in and around a small grove of pine trees near cultivated fields, but without cultivated barley. This is also a disturbed plant community, with profuse stands of wild barley. Har'el is about 50 km northeast of Ashkelon.

Spatial distribution of the niches sampled at the Israeli locations. Both the Ashkelon and Har'el locations include many separate niche environments. The isolates were sampled from different niches in approximately equal proportions to obtain representative samples for both locations.

Nineteen niche sites at Ashkelon covered a 250-m transect from the cliffs of the

coast toward the east. For data analyses, these niches were clustered in four groups according to topography and vegetation. The clusters were: **a** (the one closest to the cliffs), **b**, **c**, and **d** (the most eastern collection site). The tallest plants in the area were trees 5 m high in the east, and bushes 1 to 2 m high scattered throughout the rest of the area.

The 17 to 20 niche environments at Har'el were dispersed within and around a small pine wood comprising tall trees. Exchange of conidia among niches was, therefore, quite limited. For analyzing data, these niche sites were clustered in four groups. Cluster **b** included niches among the trees at the northern fringe of the small forest. Cluster **d** included niches at the southern fringe of the wood. This distribution has some importance in understanding the data because shading increases the development of *B. graminis* f. sp. *hordei* on barley.

Sampling Israeli populations. *B. graminis* f. sp. *hordei* isolates were collected before the peak of powdery mildew development. Three populations of the pathogen were sampled from wild barley. Five to eight single-colony isolates were collected from leaves in each of 19 niche sites at Ashkelon on 10 February 1997

(total of 108 isolates), four to six isolates from each of 20 niches at Har'el, on 11 February 1997 (total of 104 isolates), and four to eight isolates from each of 17 niches of Har'el on 15 March 1999 (total of 97 isolates).

Multiplication of inoculum from Israeli populations. The collected leaf segments with individual *B. graminis* f. sp. *hordei* colonies that had developed from natural infections were placed in petri dishes with 0.6% water agar and 35 ppm benzimidazole, then incubated for 1 day at $20 \pm 2^\circ\text{C}$ under artificial light (cool-white fluorescent lamps providing $12 \text{ h light at } 15 \pm 5 \mu\text{mol m}^{-2} \text{ s}^{-1}$). Conidia from each colony were shaken onto two leaf segments, 25 mm in length, excised from the central part of healthy, fully expanded primary leaves of the cultivar Omer, a local old cultivar, used as universal suspect in Israel, and placed in closed plastic boxes $38 \times 57 \times 15 \text{ mm}$ with 0.6% water agar and 35 ppm benzimidazole. Inoculated leaf segments were incubated under similar conditions for 13 to 18 days.

Location and sampling of Czech *B. graminis* f. sp. *hordei* populations. Conidia originating from cultivated barley fields were randomly sampled from the atmosphere by means of a mobile version of a jet spore sampler (37), which contained detached leaves in petri dishes with water agar. Susceptible barley line B-3213 (13) was used in 1997, and cv. Pallas was used in 1999 (30). Dishes were placed in the base of the spore sampler during sampling and then kept under laboratory conditions for 10 to 11 days ($20 \pm 3^\circ\text{C}$ under 8 h artificial light at $25 \pm 5 \mu\text{mol m}^{-2} \text{ s}^{-1}$) to allow collected conidia to germinate. Conidia were sampled across the Czech Republic on 29 and 31 May 1997 and on 23 to 27 May 1999.

Population designation. The Ashkelon population of 1997 and the Har'el populations of 1997 and 1999 were abbreviated as A-97, H-97, and H-99, respectively. The Czech populations of 1997 and 1999 were denoted CZ-97 and CZ-99.

Differential sets. The set of differentials used in 1997 (Table 1) was comprised of barley cv. Pallas and 15 near-isogenic 'Pallas' lines containing different genes for resistance to powdery mildew (23,30). In 1999, the set consisted of seven near-isogenic 'Pallas' lines, breeding line SG S-185, and cv. Nigrate, as well as 14 other lines carrying "new" genes conferring resistance to powdery mildew and derived from wild barley (20,21,23,24,36).

Production of differential leaf segments. About 25 seeds of each differential were sown in a pot (90 mm upper diameter) filled with sand. Plants were grown in a greenhouse at $24 \pm 2^\circ\text{C}$ for 13 to 18 days. Leaf segments 25 mm long were cut from the central part of healthy, fully expanded primary leaves. Three leaf segments of each differential were placed in a

Table 1. Set of barley differential lines, their genes for resistance to *Blumeria graminis* f. sp. *hordei*, and virulence frequency (%) found at Israeli locations Ashkelon (A) in 1997 and Har'el (H) in 1997 and 1999 and in the Czech Republic (CZ) in 1997 and 1999

No.	Differential line ^a	Resistance gene(s)	A-97 (%)	H-97 (%)	CZ-97 (%)	H-99 (%)	CZ-99 (%)
1	P01	<i>Mla1</i> , <i>Mla12</i>	99	87	42	88	43
2	P02	<i>Mla3</i>	88	96	19	91	19
3	P03	<i>Mla6</i> , <i>Mla14</i>	86	95	84	82	86
4	P08B	<i>Mla9</i>	78	69	69	58	56
5	P19	<i>Mlp1</i>	96	96	0	100	0
6	P20	<i>Mlat</i>	85	97	67	100	82
7	P21	<i>Mlg</i> , <i>MICP</i>	4	9	96	16	99
8	P04B	<i>Mla7</i> , <i>MlaNo3</i>	0	2	100		
9	P10	<i>Mla12</i> , <i>MlaEm2</i>	97	98	77		
10	P14	<i>Mlra</i>	99	100	100		
11	P17	<i>Mlk1</i>	98	100	74		
12	P11	<i>Mla13</i> , <i>MlaRu3</i>	0	0	51		
13	P15	<i>MIRu2</i>	100	100	98		
14	P23	<i>MILa</i>	100	100	89		
15	P24	<i>Mlh</i>	100	100	100		
16	Pallas	<i>Mla8</i>	100	100	100		
17	RS170-47 × Kiebitz ¹	<i>Mla17b</i>				48	0
18	RS20-1 × Kiebitz ¹	<i>Mla18</i>				37	0
19	Diamant ² × 1B-86B	<i>Mla19</i>				93	0
20	RS145-39 × Kiebitz ¹	<i>Mla20</i>				99	0
21	Diamant ² × 1B-20	<i>Mla26</i>				99	0
22	RS1-8 × Piccolo ³	<i>Mla27</i>				10	0
23	Diamant ² × 1B-151	<i>Mla28</i>				98	0
24	RS110-4 × Sonja ⁴	<i>Mla29</i>				92	0
25	Nigrate	<i>Mla30</i> , <i>Mlni</i>				79	0
26	RS142-29 × Dura ⁵	<i>Mla32</i>				97	0
27	RS42-6 × Oriol ⁶	<i>mlt1</i>				93	0
28	SG S-185	<i>mlo</i>				0	0
29	RS137-28 × Elgina ⁷	<i>Mlf1</i>				0	0
30	RS42-8 × Oriol ⁶	<i>Mli</i>				0	0
31	RS170-10 × Piccolo ³	<i>Mla25</i>				100	0
32	HSY-78 × Aramir ⁸	<i>Mli</i>				100	0
No. of isolates			108	104	101	97	275

^a Resistance genes in parental cultivars: ^{1,6,7}*Mla7*, *Mlg*; ²none; ^{3,8}*Mla12*, *Mlg*; ⁴*Mlra*; ⁵*Mlra*, *Mlu* (3).

^b Underlined genes are regarded in this contribution as "new" resistance genes.

plastic box (80 × 125 × 15 mm) on 0.6% water agar with 35 ppm benzimidazole for testing each isolate.

Inoculation of differential leaf segments. In Israel, inoculation was performed in a metal tower measuring 85 × 130 mm at the base and 350 mm high. For each isolate, a box with leaf segments from the differential set was placed at the bottom of the tower. Inoculum of each isolate collected from leaf segments was shaken onto a square piece (30 × 30 mm) of black paper to control the amount of conidia, and blown through a hole in the side of the upper part of the inoculation tower. Inoculum density was ca. 15 conidia mm⁻². The boxes with inoculated leaf segments were incubated for 10 days in an incubation room at 20 ± 2°C under artificial light (cool-white fluorescent lamps providing 12 h light at 15 ± 5 µmol m⁻² s⁻¹).

Each colony that developed from conidia collected in the Czech Republic typically produced 15,000 to 25,000 conidia. The conidia of one colony were drawn into a replaceable tip of a varipipette AW 1000 set to 0.4-ml volume and then blown out of the tip into a micro-settling tower using a syringe (10 ml) and small tube. A dish with leaf segments from the differential set was at the bottom of the micro-settling tower. Inoculum density was about 3 conidia mm⁻². Inoculation of leaf segments from differentials 17 to 32 (Table 1) in 1999 was carried out separately using five bulk isolates containing 23 to 59 colonies that developed from collected conidia. Dishes with inoculated leaf segments were kept under laboratory conditions (20 ± 3°C under 8 h artificial light at 25 ± 5 µmol m⁻² s⁻¹).

Virulence determination. Reaction type (RT) produced by each differential and *B. graminis* f. sp. *hordei* isolate combination was scored 8 or 9 days after inoculation on a 0 to 4 scale (40). This scoring scale was supplemented with RT 0(4) (i.e., RT 0 with a few RT 4 colonies) (22), which is characteristic for barley lines carrying the *mlo* resistance gene. Isolates that produced RT 3 or greater on a differential were considered virulent on the corresponding resistance gene(s). In cases where virulence was observed on a differential cultivar with two resistance genes, we concluded that the isolate had two corresponding virulence genes. But when an isolate was avirulent, one or both could be genes for avirulence. Thus, when scoring the frequencies in a population of such a virulence gene, the number of isolates virulent on the two combined resistance genes was deduced to be the least number of isolates with this particular virulence gene. Some isolates that were avirulent on the differential with two resistance genes may have had one but not both of the required virulence genes, so the actual frequency of a gene for virulence may have been greater than the calculated minimum frequency for that virulence.

Pathotype designation. The isolates were assigned numerical designations based on their virulence to matching resistance genes in the differentials 1 to 7 (Table 1). The differential set was divided into three groups: two triplets of differentials and one single differential. Each of the first two digits indicates virulence or avirulence on the three differentials of the respective triplet. If a virulence to the corresponding resistance gene is detected, the first differential line has the value 1 (2⁰), the second line has the value 2 (2¹), and the third line has the value 4 (2²). Therefore, each digit can have a value from 0 (no virulence to any of the three differential lines) up to 7 (=1 + 2 + 4, virulent on each of the three differential lines). The last digit of the designation can be only 0 or 1. The resulting number based on seven differentials defines the virulences of the isolates and consequently their classification as pathotypes (16,31,32) (Table 2).

Data analysis. Descriptive parameters of populations (virulence frequency, viru-

lence complexity, number of pathotypes, abundance, richness, and evenness) were calculated for the isolates with the HaGis program (18). In general terms, abundance signifies the frequency of the predominant pathotype (%); richness defines the number of pathotypes identified as a proportion of the number of isolates tested; and evenness reflects the variation in frequencies of individual pathotypes, whereby a more even distribution of pathotypes results in a higher value of evenness. The relative virulence complexity was determined as virulence complexity per differential (29), and its average values per isolate also were computed.

Diversities within populations and distances between populations were measured for the seven populations by two types of indices based either on pathotype frequencies (the Shannon normalized diversity and the Rogers distance) or on both pathotype and virulence structure (the Kosman diversity and distance). Because of the importance of possible associations between loci

Table 2. Pathotypes of *Blumeria graminis* f. sp. *hordei* found on wild barley at Israeli locations Ashkelon (A) in 1997 and Har'el (H) in 1997 and 1999, their virulence complexity, and number of isolates

Pathotype ^{a,b}	Virulence complexity ^c	Number of isolates			
		A-97	H-97	H-99	Total
770	6	50	47	29	126
760	5	17	22	21	60
771	7	4	6	10	20
370	5	7	2	7	16
670	5		9	5	14
360	4	3	1	7	11
570	5	6	3	2	11
730	5	9	1		10
761	6		2	4	6
660	4		2	3	5
260	3		2	1	3
560	4	1		2	3
571	6		1	2	3
160	3	1		1	2
270	4	1		1	2
460	3			2	2
530	4	2			2
710	4	2			2
740	4		2		2
750	5		2		2
170	4	1			1
320	3	1			1
350	4	1			1
510	3	1			1
520	3	1			1
630	4		1		1
720	4		1		1
No. of isolates		108	104	97	309
No. of pathotypes		17	16	15	27
C/I ^d		5.36	5.49	5.35	5.40
C/P ^e		4.29	4.88	4.67	4.33
C/D ^f		0.766	0.784	0.764	0.771

^a Classification of pathotypes is based on isolate virulence for seven differential lines (nos. 1 to 7 in Table 1).

^b Detailed data about pathotypes determined on all differentials used in a particular year are available from the first author.

^c Number of virulences.

^d Average virulence complexity per isolate.

^e Average virulence complexity per pathotype.

^f Average of relative virulence complexity is defined as the average virulence complexity per isolate per differential.

in *B. graminis* f. sp. *hordei* populations, indices based only on virulence frequencies were not considered. The Shannon normalized index of diversity within a population was first used in plant pathology by Andrivon and de Vallavieille (1) and was defined as follows:

$$Sh = \frac{-\sum_{i=1}^n p_i \ln p_i}{\ln N}$$

where the numerator is the Shannon entropy index (38), n = total number of pathotypes, p_i = pathotype frequencies ($i = 1, 2, \dots, n$), and N = sample size. The Rogers distance between two populations (34) is expressed by the following formula:

$$R = \frac{1}{2} \cdot \sum_{i=1}^m |p_{1i} - p_{2i}|$$

where m is the total number of pathotypes in both populations, and p_{1i} and p_{2i} ($i = 1, 2, \dots, m$) are frequencies of pathotype i in the first and second populations, respectively. Calculations of the Kosman diversity (KW) and distance (KB) are complex, and the corresponding explanations can be found in Kosman (27), where these parameters were denoted as Ko and K , respectively.

All diversity and distance parameters were calculated using the bootstrap method across isolates (4). One hundred fictional samples for each population were created by the resampling process. Each fictional sample consisted of 100 isolates, which were drawn independently with replacement from an original population. The values of all diversity indices and distances were calculated for each fictional sample and pair of fictional samples, respectively. The mean of the 100 values obtained for each index/distance was considered as the corresponding index of diversity within a population or distance between populations. Differences between these means were deduced to be statistically significant at $\alpha = 0.05$ using the Student t test. All diversity parameters were computed using the KOIND package (28,35).

The Fisher's exact test for contingency tables (39) was used for measuring association (disequilibrium) between pairs of virulences. The corresponding ϕ coefficient of association between pairs of differentiating characters (formula 17.5 in Sokal and Rohlf [39]) was calculated using NTSYSpc package, version 2.1 (Exeter Software, Setauket, NY). In addition, the measure of correlation between pairs of differentials (loci) was also estimated (formula 1 in Kosman [29]) using the KOIND package.

Parameters for comparison of all populations were calculated on the basis of virulence patterns of isolates on the set of the following seven differential lines: P01, P02, P03, P08B, P19, P20, and P21 (Table 1).

RESULTS

The differentials used, their genes for resistance to powdery mildew, and frequencies of corresponding virulences found in the populations that were studied are presented in Table 1.

Israeli *B. graminis* f. sp. *hordei* populations. *Ashqelon* 1997. All 108 isolates contained virulences *Ru2*, *La*, *h*, and *a8* (Table 1). In contrast, no virulences were found against the combined resistances *Mla7*, *MlaNo3* or *Mla13*, *MlaRu3*, which were present in the differential lines P04B and P11, respectively, and the frequency of virulence to the combination *Mlg*, *MICP* (P21) was only 4%. The virulence frequency detected on the other nine differentials ranged from 78 to 99%.

Har'el 1997. All 104 isolates exhibited virulences *ra*, *k1*, *Ru2*, *La*, *h*, and *a8* (Table 1). Virulence to the combined *Mla13*, *MlaRu3* (P11) was not detected. We found two isolates (2%) virulent on P04B (*Va7*, *VaNo3*) and nine isolates virulent on P21 (*Vg*, *VCP*). The virulence frequency detected on the other seven differentials ranged from 69 to 98%.

Ashqelon and Har'el, 1997. Frequencies of virulence among isolates from *Har'el* in 1997 were generally similar to those at *Ashqelon*. The greatest differences were that the frequency of virulence to the combined *Mla1*, *MlaAl2* was 12% lower and the frequency of virulence to *Mlat* was 12% greater at *Har'el* than at *Ashqelon* in 1997.

Twenty-six pathotypes were detected among the 212 isolates from *Ashqelon* and *Har'el* in 1997 (Table 2). The eight most frequent pathotypes represented 88.2% of all isolates. Eleven pathotypes were each represented by only one isolate. The predominant pathotype 770 comprised 46.3% of the isolates from *Ashqelon* and 45.2% of the isolates from *Har'el*. Two other pathotypes found abundantly at *Ashqelon* (760 and 771) also occurred very frequently at *Har'el*. Pathotype 771 contains all seven virulences that were identified. Nineteen of the pathotypes were unique to either *Ashqelon* (10 pathotypes) or *Har'el* (9 pathotypes). Of these, the most abundant pathotype, designated 670, comprising nine isolates, was detected only at *Har'el*. Average complexity at *Ashqelon* (5.36) was slightly lower than at *Har'el* (5.49). In both populations, pathotypes with the lowest complexity were quite rare.

Har'el 1999. No matching virulence was found for the resistance gene *mlo* (*Vo*) originating from barley, or resistance genes *Mlf1* (*Vf1*) and *Mli* (*Vi*) derived from wild barley (Table 1). In contrast, all isolates had virulences *Vp1* and *Vat* as well as virulence against resistance genes *Mla25* (*Va25*) and *Mlj* (*Vj*) derived from wild barley. Virulence frequency to the other 16 mildew resistance genes ranged from 10 to 99%. Based on the first seven differentials,

15 pathotypes were detected among 97 isolates (Table 1). The most frequently recorded (29 isolates) was again pathotype 770 (Table 2).

Har'el 1997 and 1999. Frequencies of virulence to differentials 1 to 7 at *Har'el* in 1999 were generally similar to those in 1997. The greatest differences were that the virulence frequency to the combined *Mla6*, *Mla14* was 13% lower, and the virulence frequency to the combined *Mlg*, *MICP* was 7% greater in 1999 than in 1997.

Comparison of Israeli and Czech *B. graminis* f. sp. *hordei* populations. In 1997 and 1999, 309 isolates were obtained for study from three populations of *B. graminis* f. sp. *hordei* on plants of wild barley at two Israeli locations and 376 isolates were obtained for study from two Czech populations (CZ-97 and CZ-99) (Table 1). The data on pathotypes and their frequencies in the Czech populations are not presented here, but several parameters of populations and diversities were calculated for comparing with the Israeli populations (Table 3).

Out of a total of 27 pathotypes found in Israel, six of the eight most frequent pathotypes were found in all three populations comprising 244 isolates (79.0%) (Table 2). There were 126 isolates (40.8%) belonging to pathotype 770, which was the most abundant in all three populations (29.9 to 46.3%). No isolate of pathotype 670 was found at *Ashqelon*, whereas 14 such isolates were found at *Har'el* in both years. Nine isolates of pathotype 730 were found at *Ashqelon* in 1997, whereas only one isolate of 730 was found at *Har'el*.

Thirty-four pathotypes were found in the Czech Republic. Twenty-one pathotypes were found in both Czech populations, and 82 isolates (21.8%) belonged to the most abundant pathotype 451. No common pathotype among the Israeli and the Czech populations was found.

Average virulence complexity per isolate (C/I) of the three Israeli populations (Table 3) was high and very similar (5.35 to 5.49 out of a possible seven maximum), while those for the two Czech populations were lower (3.77 and 3.84). Richness of the three Israeli populations was also very similar and quite low (0.154 to 0.157), while those for one Czech population was relatively high (0.238) and the other was very low (0.113). Evenness of populations A-97 and H-97 was nearly identical, whereas evenness of population H-99 was higher. The evenness of population CZ-99 was similar to that of H-99, but evenness for CZ-97 was much greater.

The Shannon normalized (Sh) and the Kosman (KW) diversity indices were significantly different for the five populations (Table 3). The highest and the lowest diversities according to the indices were recorded for populations CZ-97 and H-97, respectively. The diversity of the Czech

populations was much higher than that of the Israeli populations. The diversity for the CZ-97 population was higher than for CZ-99. The parameters for the populations of Ashkelon and Har'el in 1997, on the basis of 11 differentials, were similar. The diversities calculated on the basis of the reactions of 11 differentials were smaller compared with those based on the reactions of seven differentials.

Both the Rogers' (*R*) and Kosman's (*KB*) indices of distance between populations were significantly different for the six comparisons (seven differentials) (Table 4). Highest distances according to both indices were recorded between populations H-97 and CZ-97 and between H-99 and CZ-99, and the lowest distance was between H-97 and H-99. The Kosman distance between A-97 and H-97 calculated for 11 differentials was smaller than the Kosman distance calculated for seven differentials, but the Rogers' distance calculated for 11 differentials was greater than that for seven differentials.

Spatial distribution of diversity within Israeli locations. The diversity for clusters of niches at one location, Ashkelon, showed an interesting feature (Table 5). Diversity was the lowest in the cluster of niches near the cliffs (**a**) and increased toward the eastern cluster of niches (**d**). Diversity of the whole sample from all four clusters of niches was much higher than in the cluster near the cliffs, but lower than in the cluster in the east. At Har'el, the results were not consistent. In 1997, the diversity was larger in the northern fringe of the grove [cluster (**b**)] where it is cooler and more shaded; both these conditions favor *B. graminis* f. sp. *hordei* development compared with the southern fringe [cluster (**d**)], where it is warmer and sunnier. But in 1999, the situation was the reverse. However, the differences in pathotypes between the northern cluster and the southern cluster are quite marked, indicating that isolation by a stand of trees is quite effective.

DISCUSSION

Virulence frequencies. Long-term temporal changes in virulence in *B. graminis* f. sp. *hordei* populations can be examined by comparing current results with those from a previous study on virulence that also included isolates from Har'el as well as four other locations in Israel. Among the 201 isolates from Har'el examined in the current study, we found frequencies of virulence to 12 resistance genes (*Mla1*, *Mla3*, *Mla6*, *Mla7*, *Mla8*, *Mla9*, *Mla12*, *Mlat*, *Mlk1*, *MILa*, *Mlra*, and *MIRu2*) similar to those reported by Dinooor and Eshed 18 to 20 years earlier for the 63 isolates from this location in 1979–1980 that they studied (5). The frequencies of virulence to *mlo*, *Mlg*, and *Mla13* in our study, however, differed from those found by Dinooor and Eshed. Although we found no isolates virulent to *mlo*, Dinooor and Eshed reported that 5% of their isolates from Har'el were virulent on one differential line with *mlo*, and 35% were virulent on a second line with *mlo*. They pointed out, however, that isolates that appeared virulent on *mlo* in the virulence test could not be propagated on barley lines with *mlo*. It is possible, therefore, that the apparent virulence of these isolates may have been due to some environmental factors causing breakdown of the resistance in some of the differential plants with *mlo*.

We found that 9% of the isolates from Har'el were virulent on *Mlg* lines in 1997, and 16% were virulent in 1999. Dinooor and Eshed (5) reported that 32% of their isolates from Har'el were virulent on *Mlg* lines. We did not find any virulence on the differential with combined resistance genes *Mla13*, *MlaRu3* at either Har'el or Ashkelon, whereas Dinooor and Eshed found 25% of their isolates were virulent on 'Rupée', which contains *Mla13* and *MlaRu3* plus two other resistance genes. They also found that 38% of their isolates were virulent on 'Vagabond', which has *Mla13* (15). Possible reasons for the large decline in frequency of virulence to *Mlg* and to *Mla13* and *MlaRu3* between 1979 and 1997 are not obvious. Cultivated barley is a minor crop in Israel, and furthermore, *Mla13* was not introduced into Israeli barley cultivars. Our isolates were obtained from a site at Har'el a few hundred meters away from the site sampled by Dinooor and Eshed (5), because host plants at their site had in the meantime been eliminated by herbicide treatments. There were no physical barriers to spore movement between the site sampled in 1979 and the southern niche of the 1997 site, as this land had been planted to wheat, corn, or cotton, or left fallow. Tall trees likely created a barrier to spore movement between

Table 4. Distance between Israeli [Ashkelon (A) and Har'el (H)] and Czech (CZ) populations of *Blumeria graminis* f. sp. *hordei* in 1997 and 1999

Populations	No. of differentials	Distance between populations	
		Kosman, <i>KB</i> ^a	Rogers, <i>R</i> ^b
A-97 and H-97	11 ^c	0.059	0.334
A-97 and H-97	7 ^d	0.080	0.302
A-97 and H-99	7	0.098	0.381
H-97 and H-99	7	0.066	0.296
H-97 and CZ-97	7	0.503	1.000
H-99 and CZ-99	7	0.458	1.000
CZ-97 and CZ-99	7	0.084	0.372

^a (27).

^b (34).

^c No. 1 to 11 in Table 1.

^d No. 1 to 7 in Table 1.

Table 3. Comparison of Israeli populations of *Blumeria graminis* f. sp. *hordei* found at Ashkelon (A) in 1997 and Har'el (H) in 1997 and 1999, and the Czech (CZ) populations of 1997 and 1999

Parameter	Population						
	A-97	A-97	H-97	H-97	H-99	CZ-97	CZ-99
Number of isolates	108	108	104	104	97	101	275
Number of differentials concerned ^a	7	11	7	11	7	7	7
Number of pathotypes	17	20	16	19	15	24	31
Number of pathotypes with at least two isolates	9	9	11	11	12	16	22
Abundance ^b	46.3	45.4	45.2	45.2	29.9	23.8	21.1
Average virulence complexity per isolate, C/I	5.36	8.29	5.49	8.49	5.35	3.77	3.84
Average of relative virulence complexity, C/D	0.766	0.754	0.784	0.772	0.764	0.539	0.549
Richness (number of pathotypes/number of isolates)	0.157	0.185	0.154	0.183	0.155	0.238	0.113
Evenness	0.674	0.671	0.666	0.656	0.797	0.856	0.789
Shannon normalized index of diversity, <i>Sh</i> ^c	0.398	0.415	0.364	0.410	0.459	0.847	0.568
Kosman's index of diversity, <i>KW</i> ^c	0.205	0.138	0.187	0.132	0.290	0.467	0.412

^a Used for pathotype formulas and calculations of parameters.

^b Frequency of the predominant pathotype (%).

^c Diversity indices were calculated by the KOIND package (28,35) as averages on the basis of the bootstrap subsamples; the other indices were calculated by the HaGis program (18) on the original sample of the isolates.

the northern niche sampled in 1997 and the 1979 site as well as the southern niche of 1997. On the other hand, we did not find virulence to the combination *Mla13*, *MlaRu3* at either Har'el or Ashquelon, which is about 50 km from Har'el. It is doubtful, therefore, that a few hundred meters between the Har'el site sampled by Dinoor and Eshed and our site could account for the large difference in frequency of virulence to *Mla13* and *MlaRu3*. Another possible explanation is that these resistance genes may have been present in the wild barley population at Har'el at a higher frequency in the 1970s than in the 1990s.

In the Israeli populations, we found considerably higher frequency of virulences for most resistance genes common in European barley cultivars (*Va1*, *Va3*, *Va6*, *Va9*, *Va12*, *Va13*, *Vk1*, *Vat*, and *VLa*) (3) compared with the European populations (19,42), and also a high frequency of virulence *Vp1*, which is rarely recorded in Europe. In contrast, the frequency of the combined virulences *Va7*, *VaNo3* and *Vg*, *VCP* were markedly lower in the three Israeli populations than in those from Europe (19).

The seven differentials that were selected to compare the five populations (three Israeli and two Czech ones) could potentially distinguish 128 (i.e., 2^7) pathotypes. We found 27 pathotypes in the Israeli populations and 34 in the Czech populations, but no pathotype was common to both countries. The largest dissimilarity among pathotypes was revealed by the differential line P19 with resistance gene *Mlp1*. The frequency of the virulence *Vp1* in the Israeli populations was 96 to 100%, whereas this virulence was never found in the Czech populations. The most likely explanation is that the gene *Mlp1* was not used in the breeding of any commercial European cultivar.

In 1999, we used differentials mostly from sources carrying "new" wild barley resistance genes against powdery mildew. These sources, originating from collections of wild barley in Israel, are now widely used, particularly by European breeders for developing barley cultivars resistant to powdery mildew. However, the frequency

of virulences for these genes in the Israeli populations of the pathogen has not previously been determined. In 1999, we recorded a very high frequency of virulences for nine of these 14 resistance genes at Har'el, and a moderate frequency of virulences for three others. No isolate overcame the resistance genes *Mlf1* and *Mli*. However, the virulence frequencies of *Vf1* and *Vi*, and also *Va27* could be reduced by adopting other resistance genes such as *Mla7* and/or *Mlg*, which may be present in the corresponding differentials (Table 1).

Some virulences, e.g., *Vg*, *Va7*, and *Va13*, which are abundant in Europe (19), are absent or of low frequency in Israel. This suggests that the corresponding resistance genes in the host populations of wild barley in Israel could be present in low frequencies or absent. On the other hand, the high virulence frequencies found in the Israeli populations for the resistance genes *Mla1*, *Mla3*, *Mla9*, *Mlat*, *Mla12*, *Mla*, *Mlk1*, *MIRu2*, *MILa*, *Mlh*, and *Mla8* suggest that these genes, derived from landraces, could also be present with others in the populations of wild barley in Israel.

A few correlated genes and weak associations between virulences for the set of seven old differentials were discovered in five of the investigated populations. However, the observed correlations and associations were not consistent between 1997 and 1999. Indeed, the two parameters, ϕ -coefficient of association and the measure of correlation, do not confirm sustainable associations between virulence genes in either the Har'el or the Czech populations.

Characteristics of the populations.

According to the calculated distances, H-97 and H-99 populations are the most similar (same location in different years), and A-97 and H-99 populations are the most dissimilar (different locations in different years) (Table 4). However, relative dissimilarities between the pairs of populations differ and depend on which index is used. The Rogers' genotypic distance (*R*), which is based on the frequencies of pathotypes in the population, was least able to differentiate between pairs of populations, but it does not take into account degrees of similarity between the pathotypes. Using this index, A-97 and H-97

populations, and H-97 and H-99 populations were found to be almost equally dissimilar (the values of the Rogers' distance are 0.302 and 0.296, respectively) despite the different reasons for the dissimilarity. H-97 and H-99 populations were dissimilar because the frequencies of most of the 11 common pathotypes were different (Table 2). On the other hand, A-97 and H-97 populations were dissimilar because they shared just seven pathotypes, but the frequencies of the dominant pathotypes 770 and 760 were similar (Table 2). The Rogers' distance between A-97 and H-97 populations was higher for the set of 11 differentials compared with the set of seven (Table 4), due to the larger number of pathotypes discovered within these populations after testing the 11 differentials (Table 3).

According to the Kosman genetic distance (*KB*), the dissimilarity between two populations from distant locations in the same season (A-97 and H-97) was 20% larger (0.080) than that between two populations from the same location in two different years (H-97 and H-99; 0.066). This suggests that frequencies of virulence may be relatively stable within populations of *B. graminis* f. sp. *hordei* on wild hosts in Israel and that migration between distant populations is limited. In comparing the genetic distance among the three Israeli populations, we may conclude that the populations in different years at the same location are more similar than the populations of two locations in the same year. As expected, the genetic distance between populations of different countries was much larger than the distance between populations within each country.

The Kosman index of genetic diversity (*KW*) was much smaller for 11 differentials than for seven differentials despite the fact that each additional differential could result in increasing diversity. The data in Tables 1 and 2 show that three of the additional differentials (nos. 9 to 11) are susceptible to most isolates and one (no. 8) is resistant to most isolates. Indeed, they differentiate only slightly among the isolates, and therefore, the addition of these four cultivars hardly changes the "absolute" diversity (diversity *per se* expressed

Table 5. Diversity in populations of *Blumeria graminis* f. sp. *hordei* in niches within two Israeli locations, Ashquelon (A) in 1997 and Har'el (H) in 1997 and 1999

Index	Niches and populations (shaded)										
	A-97a ^a	A-97b ^a	A-97c ^a	A-97d ^a	A-97	H-97b ^b	H-97d ^b	H-97	H-99b ^b	H-99d ^b	H-99
<i>KW</i> ^c	0.149	0.194	0.226	0.259	0.205	0.235	0.140	0.187	0.228	0.295	0.290
<i>Sh</i> ^d	0.293	0.295	0.389	0.432	0.398	0.367	0.303	0.364	0.358	0.463	0.459
No of isolates ^e	32	22	29	25	108	37	31	104	36	45	97

^a Four clusters of niches at Ashquelon, along a transect from the cliffs above the beach (a) to the east (b, c, and d; d is the most eastern).

^b Two niches on both sides of a grove at Har'el.

^c Kosman's index of diversity within population. Diversity indices were calculated by the KOIND package (28,35) as averages on the basis of the bootstrap subsamples.

^d Shannon's normalized index of diversity. Diversity indices were calculated by the KOIND package (28,35) as averages on the basis of the bootstrap subsamples.

^e Total number of isolates included in the corresponding test.

on seven differentials). However, the Kosman index measures diversity per differential, i.e., the “absolute” diversity divided by the number of differentials (11 and 7, respectively). This explains why the *KW*-diversity measure produces lower values of diversity for the given set of 11 differentials than the set of seven, and why the values of Kosman’s genetic distance (*KB*) between populations have also decreased greatly by including these additional four differentials in calculating the distance index (Table 4).

According to Groth and Roelfs (17), a pathogen population is more diverse if: (i) that population consists of a larger number of phenotypes for a given number of isolates; (ii) it is characterized by an even distribution of phenotypes; or (iii) the number of differences in virulence between phenotypes is large. Properties (i) and (ii) are defined by the richness and evenness parameters, respectively, and the Shannon normalized index (*Sh*) takes into account both these properties (25). Increasing the number of differentials should result in an increase in the number of pathotypes detected in the population and, therefore, an increase in the measure of population richness. If the population evenness remains constant, the Shannon normalized index (*Sh*) should also increase as the number of differentials increases. Both trends were seen in this study, and this explains the higher values of the Shannon normalized index calculated from 11 differentials compared with seven (Table 3). Property (iii) may be expressed by the measure of average differences among isolates, where dissimilarity is measured by the simple mismatch coefficient.

Our expectation was that the diversity of *B. graminis* f. sp. *hordei* in Israel, which reflects the diversity of wild barley, would be larger than in Europe (e.g., the Czech Republic), where uniform host genotypes are grown over a large area. The findings were, however, quite different; both Czech populations were much more diverse than the Israeli populations as revealed by the average dissimilarity between the virulence patterns of isolates, property (iii) of Groth and Roelfs (17) (Table 3). Differences in virulence between isolates in the Czech populations were larger compared with the Israeli populations due to the virulence–avirulence ratio observed on differentials 1, 2, and 6 (Table 1). If one considers diversity with respect to any differential (33), then it is natural to assume that: (i) minimum diversity is observed when all isolates are either virulent or avirulent on this differential (the virulence–avirulence ratio equals infinity or 0, respectively), and (ii) maximum diversity is observed when the numbers of virulent and avirulent isolates are equal (the virulence–avirulence ratio equals 1). Comparison of the Israeli and the Czech populations of *B. graminis* f. sp. *hordei* with

respect to differentials 1, 2, and 6 revealed that the virulence–avirulence ratios were considerably closer to 1 for the Czech populations (Table 1), thereby indicating high diversity. For example, the virulence–avirulence ratio for P01 for the Czech CZ-97 population was 0.724 (42/58), whereas for the Israeli H-97 population it was 7.25 (87/13).

Evolutionary aspects. An important evolutionary force acting in pathogen populations is direct selection of virulences to resistance genes present in the host in a given area (9). Powdery mildew resistance genes present in barley cultivars grown in the Czech Republic were described in detail (12), and also the relative popularity of these cultivars over several years has been documented. Most wild barley accessions collected in Israel carry at least one resistance gene (10). Our recent investigations were aimed mainly at identifying sources possessing fully effective resistances (11). However, many other resistance genes, probably including well-established alleles, were detected (10).

The lower diversity in the Israeli pathogen populations, relative to the Czech populations, is due to the complete adaptation of virulence to resistance, which has probably reached its peak in Israel. Israel is part of the center of diversity of wild barley, and the Israeli host population is highly diversified and a rich source of genes conferring resistance to powdery mildew (14). Long-term natural selection in the Israeli populations of *B. graminis* f. sp. *hordei* has increased the frequencies of most virulence alleles to almost 100% and has created a center of virulence complexity of the pathogen.

Increasing virulence frequency is associated with rising complexity of the pathogen populations and evolution of so-called “super-races”, which are virulent against a number of resistance genes. Our results demonstrate that such complex pathotypes are abundant in nature and confirms the effect of evolutionary forces on pathogen development even where there is high host and nonhost diversity.

Six virulences (*Va1*, *Va3*, *Va6*, *Va9*, *Vat*, and *Vg*) studied in 1997 and 1999 and at least five other virulences (*Va7*, *Va12*, *Va13*, *Vk1*, and *VLa*) studied in 1997, which were effective against “old” resistance genes, came under intensive selection pressure in Europe, particularly in the Czech Republic, due to widespread growth of cultivars with the corresponding resistance genes (9). The adaptation of the Czech pathogen population also resulted in a huge increase in its virulence complexity against 11 corresponding resistance genes, and ranged from approximately 0.90 in 1971 to 7.89 in 2001 (8). However, virulence evolution in the Czech Republic has not advanced as far as in Israel, thereby maintaining higher diversity in the Czech pathogen population.

Based on the number of virulence genes that have already developed in *B. graminis* f. sp. *hordei*, it can be assumed that if newly discovered resistant genes are used individually in a new cultivar, this resistance will eventually, and likely quickly, be overcome due to spontaneous mutations in the pathogen population followed by selection on the new resistant cultivar. If several new resistant genes are deployed together in a new cultivar (pyramiding), it is anticipated that new pathotypes of *B. graminis* f. sp. *hordei* with virulences matching the combination of resistance genes will not arise quickly because the probability that multiple random mutations will occur together, which is necessary to overcome two or more current fully effective resistance genes present jointly in one cultivar, is much less than the probability of a single mutation for virulence to one currently fully effective gene. However, this pathogen has demonstrated ability to develop complex pathotypes. Therefore, while it is recommended for breeding resistant barley cultivars to pyramid new genes that are fully effective in a given epidemiological area in order to prolong the durability of resistance, there will continue to be a need to search for new resistant genes and to monitor pathotypes in order to ensure farmers have cultivars that can effectively resist powdery mildew.

Clusters of niches at the two locations in Israel were studied to examine diversity within a location. Topography and local conditions explain the directional trend in diversity at Ashkelon and the effect of the physical barrier created by a stand of tall trees at Har’el. At Ashkelon, there is a diversity gradient increasing from the coast toward the east and corresponding to the prevailing wind, which constantly disperses pathogen spores. The data from Har’el show that diversity is significantly different on both sides of the large physical barrier (Table 5, niches H-97b versus H-97d). It could be due to localized microevolution, aided by sexual recombination, without dispersal over the barrier.

This study revealed great differences between Israeli and Czech pathogen populations in the frequency of most virulences examined, and particularly virulences for “new” resistance genes originating from Israel. It establishes that both populations belong to distinct epidemiological units with little or no transfer of *B. graminis* f. sp. *hordei* spores between them. This should stimulate the exploitation of host resistance diversity found in wild barley from Israel (10,11) in breeding barley cultivars resistant to powdery mildew in Europe and throughout the world.

It is unlikely that significant exchange of inoculum between populations of *B. graminis* f. sp. *hordei* from Central Europe and the Middle East can occur by aerial dispersal (2) because of the natural barriers (mountains, Mediterranean Sea) and the

distance between these two regions. There is, however, a risk of transferring isolates between isolated populations by human activities. As the results suggest, Israeli isolates exhibit high virulence complexity not only to most resistance genes possessed by current barley cultivars but also to new sources of resistance originating from Israel. One pathotype found at Har'el in 1999 was virulent against at least 12 "new" resistance genes. Pathotypes such as this should not be imported into Europe for research because of the serious threat to the successful long-term effort to collect, study, and utilize resistance from wild barley for developing European cultivars with durable resistance. Thus, research on such pathogenic cultures should be carried out in the area of their origin or, alternatively, plant material rather than pathogens could be brought from this area for testing (11).

The ability of *B. graminis* f. sp. *hordei* to adapt and evolve complex pathotypes, as revealed in this study, will limit the successful use of crop diversity through growing varietal mixtures for managing barley powdery mildew (41). Pyramiding resistance genes together in one cultivar is expected to be more successful. Success could be improved further with better understanding of pathogen evolution. Further research should focus on the time required for these highly complex pathotypes to evolve and to determine to what extent the adaptation process of pathogen populations has been slowed by host diversification.

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